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(के एम बी आई) — विशिष्टि

Potash Mobilizing Bacterial
Inoculant (K M B I) — Specification

ICS 65.080

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FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Soil Quality and Fertilizers Sectional Committee had been approved by the Food and Agriculture Division Council.

Bio fertilizers have been identified as alternative to chemical fertilizers to increase soil fertility and crop production in sustainable farming. Bio fertilizers are natural fertilizers which are microbial inoculants of bacteria, algae, fungi alone, or in combination, and they augment the availability of nutrients to the plants. The use of bio fertilizers, in preference to chemical fertilizers, offers economic and ecological benefits by way of soil health and fertility to farmers.

Bio fertilizers are products containing living cells of different types of microorganisms, which have the ability to convert nutritionally important elements from unavailable to available form through biological processes. They are involved in symbiotic and associative microbial activities with higher plants. These are natural mini-fertilizer factories that are economical and safer source of plant nutrition for increasing the agricultural production and improving soil fertility.

Potassium is one of the essential nutrients for plant growth and required in large amount to achieve optimum growth. A deficiency of this element, also known as potash deficiency, exhibits chlorosis (loss of green colour) along the leaf margins. Then, in severe cases, leaf will turn into yellow colour and eventually will fall off. It also affects plant growth and canopy photosynthesis process. Furthermore, the presence of extreme amounts of reduced substance in poorly drained soils will cause retarded root growth and reduced potassium uptake.

Bio fertilizer is a good platform to deliver this primary macronutrient by assistance of potassium mobilizing bacteria, which are capable of solubilizing potassium rock through production and secretion of organic acids. Thus, they are important in the formation of humus in soil, the cycling of other minerals tied up in organic matter, and the prevention of build-up of dead organic materials. These are aerobic bacteria which play an important role in maintaining soil structure by their contribution in the formation and stabilization of water-stable soil aggregates.

The potash mobilizing bacterial inoculants (KMBI) include different groups of soil micro-organisms which release immobilized potassium compounds into available form. The species of *Bacillus*, *Pseudomonas* and *Fraturia* are active in bioconversion. The most efficient bacterial isolates are identified as *Fraturia aurantia*, *Bacillus edaphicus* and *Bacillus muscilaginosus*. These efficient micro-organisms have shown capability to mobilize potash content available in soil naturally or applied in the soil through fertilizer application. Use of KMBI releases some of the gorged potassium between the layers of clay. Therefore, KMBI increase potassium availability in soils besides increasing mineral contents in plants. The potassium mobilizing bacteria is gram negative rod type bacteria, can grow in pH 3.5 to 11 and capable of mobilizing the mineral potash to the tune of 40-60 kg/ha.

Many brands of KMBI are marketed in the country and they have been found to vary in quality. Hence, the need was felt for an Indian Standard not only to test the quality of inoculants in order to provide the farmers with certified inoculants but also to help the producers to improve quality of their products.

It is obligatory on the part of the manufacturers to employ qualified soil microbiologist or microbiologist or agricultural graduates or graduates in biology trained in soil microbiology in their staff. Manufacturers shall also maintain a quality control laboratory capable of carrying out the tests according to this standard.

Committee responsible for the formulation of this standard is given at Annex D

For the purpose of deciding whether a particular requirement of this standard is complied with the final value, observed or calculated, expressing the result of a test or analysis shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

POTASH MOBILIZING BACTERIAL INOCULANT (K M B I) — SPECIFICATION

1 SCOPE

This standard prescribes the requirements, method of sampling and tests for potash mobilizing bacterial inoculants.

2 REFERENCES

The following Indian Standards contain provisions which through reference in this text constitutes provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent edition of the standards indicated below:

<i>IS No.</i>	<i>Title</i>
IS 460 (Part 1) : 2020	Test sieves — Specification: Part 1 Wire cloth test sieves (<i>fourth revision</i>)
1070 : 1992	Reagent grade water (<i>third revision</i>)
6850 : 1973	Agar, microbiological grade
6853 : 1973	Peptone, microbiological grade
7004 : 1973	Yeast extract, microbiological grade
8268 : 2020	<i>Rhizobium</i> inoculants — Specification (<i>third revision</i>)

3 TERMINOLOGY

For the purpose of this standard, the following definitions shall apply.

3.1 Potassium Mobilizing Bacterial Inoculant (KMBI) — KMBI is a product having high population of a strain(s) of bacteria intended to solubilise/mobilize insoluble potassium of the soil into readily available form to the plants.

3.2 Group of Crops — KMBI is beneficial for all types of crops in terms of mobilization of potassium present in the soil in non-available form.

4 REQUIREMENTS

4.1 KMBI shall be carrier-based, the colour depending on the colour of the carrier. Carrier material, such as peat, lignite, peat soil, humus, talc or similar material favouring growth of microorganisms may be used. It shall be neutralized with calcium carbonate and then sterilized. When tested for fineness, it shall pass through 0.15 to 0.212 mm IS sieve {see IS 460 (Part 1)}.

4.2 When tested by the method prescribed in Annex A of this standard, KMBI shall contain minimum 5×10^7 viable potassium mobilizing bacterial cells/g of the powder, granules or carrier on dry weight basis during the entire period of shelf-life from the date of manufacture, as specified on the packet.

4.3 When tested by the method prescribed in Annex A, KMBI shall have no contamination with other micro-organisms at 10^5 dilution.

4.4 When tested by the method prescribed in Annex B of IS 8268, the pH of KMBI shall be 6.5 to 7.5.

4.5 When tested by the method prescribed in Annex A of this standard, KMBI shall have minimum 10 mm solubilisation zone in prescribed media having at least 3 mm thickness.

4.6 Specified mother culture be obtained from any recognized institution maintaining the mother culture. The manufacturer may control the quality of broth as given in Annex B, it should get verified at least by two institution as mentioned below.

NOTE — At present National Centre of Organic Farming (NCOF), Ghaziabad and its six Regional Centres located at Bengaluru, Bhubaneshwar, Hissar, Imphal, Jabalpur and Nagpur; Indian Agricultural Research Institute (IARI), New Delhi; Tamil Nadu Agricultural University (TNAU), Coimbatore; University of Agricultural Science, Bengaluru are sources for supplying the mother culture.

5 PACKING, MARKING AND STORAGE

5.1 Packing

KMBI shall be packed in packaging material of low density polyethylene/polypropylene bags thickness

of which shall not be less than 75-100 micron or in suitable HDPE/polypropylene/plastic/glass bottles.

5.2 Marking

5.2.1 Each polyethylene pack shall be marked legibly and indelibly with the following information:

- a) Name of the product specially as 'POTASH MOBILIZING BACTERIAL INOCULANT';
- b) Name and address of the manufacturer;
- c) Crop(s) for which intended;
- d) Type of the carrier;
- e) Batch number;
- f) Date of manufacture;
- g) Expiry date which shall not be less than 6 months from the date of manufacture;
- h) Net quantity in kg and area meant for;
- j) Storage instructions worded as under "STORE IN COOL PLACE AWAY FROM DIRECT SUNLIGHT AND HEAT"; and
- k) Any other information required under the *Legal Metrology (Packaged Commodities) Rules, 2011*.

5.2.2 Items (c), (f) and (g) shall be printed on a coloured ink background.

5.2.3 Directions for use of KMBI shall be printed briefly on the packets as given in Annex C of this standard. A separate pamphlet may preferably be given with it.

5.2.4 BIS Certification Marking

The product(s) conforming to the requirements of this standard may be certified as per the conformity assessment schemes under the provisions of the *Bureau of Indian Standards Act, 2016* and the Rules and Regulations framed thereunder, and the products may be marked with the Standard Mark.

5.3 Storage

KMBI shall be stored by the manufacturer in a cool and dry place away from direct heat preferably at a temperature of 20 °C and not exceeding 30 °C. It shall also be the duty of the manufacturer to instruct the retailers and, in turn, the users about the precautions to be taken during storage.

6 SAMPLING

The representative samples of the KMBI shall be drawn as prescribed in Annex F of IS 8268.

7 TESTS

7.1 Tests shall be carried out by the methods prescribed in 4.

7.2 Unless otherwise specified, quality reagents, chemicals and distilled water (*see* IS 1070) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect experimental results.

ANNEX A

(Clauses 4.2, 4.3 and 4.5)

DETERMINATION OF TOTAL VIABLE COUNT AND CONTAMINATION AND ESTIMATION OF POTASSIUM SOLUBILIZATION ZONES

A-1 APPARATUS

A-1.1 Pipettes, graduated, 1 ml and 10 ml.

A-1.2 Conical Flasks, 150 ml and 250 ml.

A-1.3 Screw Capped Tubes, 10 ml.

A-1.4 Incubator

A-1.5 Petri Dishes, clear, uniform, flat-bottomed.

A-1.6 Hot Air Oven

A-1.7 Autoclave

A-1.8 pH Meter

A-1.9 Colony Counter

A-1.10 Hand Tally or Mechanical Counting Device

A-1.11 Magnifying Lens

A-2 REAGENTS

A-2.1 Use a medium of the following composition for analysis of total viable count and contamination:

Mannitol	15.0 g
Yeast extract (<i>see</i> 7004)	3.0 g
Peptone (<i>see</i> 6853)	2.0 g
Agar (<i>see</i> 6850)	18.5 g
Trace element solution	1 ml
Distilled water	1 000 ml

A-2.1.1 Trace Element Solution

Sodium molybdate	0.20 g
Boric acid	0.28 g
Manganese sulphate	0.23 g
Copper sulphate	0.01 g
Zinc sulphate	0.03 g
Distilled water	1 000 ml

A-2.2 Use a medium of the following composition for studying zone solubilization in KMBI:

Glucose	5.0 g
Magnesium sulphate	0.005 g
Ferric chloride	0.1 g

Calcium carbonate	2.0 g
Potassium mineral (mica powder)	2.0 g
Calcium phosphate	2.0
Distilled water	1 000 ml

A-2.3 Sterilization and preparation process for plates is the same as described in IS 8268.

A-3 PREPARATION OF SERIAL DILUTION FOR CELL COUNT METHOD

Dispense 10 g of KMBI to 90 ml of sterile distilled demineralized water and shake for 10 minutes on a reciprocal shaker or homogenizer. Make serial dilutions up to 10^{10} level. Pipette out 0.1 ml or suitable aliquots of 10^6 to 10^9 dilutions using sterile pipettes and deliver to the petri dishes containing set medium as described in A-2.1.

A-3.1 Spread the aliquots over the plate uniformly with a spreader or use droplet method. Invert the plates and promptly place them in the incubator at 28 ± 2 °C for 3 days. Use 3 replicates of 10^5 , 10^6 and 10^7 dilution.

A-4 COUNTING

A-4.1 Count the colonies with the aid of magnifying lens under uniform and properly controlled, artificial illumination. Use a colony counter, equipped with a guide plate and rules in centimeter square. Record the total number of colonies with the hand tally. To distinguish colonies from dirt, specks and other foreign matter, examine doubtful objects carefully.

A-4.2 Count all plates but consider for the purpose of calculation plates showing more than 30 and less than 300 colonies per plate. Disregard colonies which absorb congo red and stand out as reddish colonies. *Fraturia aurentia* (KMBI) stands out as white-opaque glistening and domed colonies. Count such colony numbers and calculate figures in terms of per litre of carrier. Also check for freedom from contamination at 10^5 dilution (*see* 4.3).

A-5 METHOD FOR ESTIMATION OF POTASSIUM SOLUBILIZATION ZONES

A-5.1 Take 10 g of KMBI in 90 ml sterile distilled water.

A-5.2 Make a ten folds dilution series up to 10^{10} .

A-5.3 Take 0.1 ml aliquot of 10^5 to 10^7 dilution using sterile pipettes and deliver to petri dishes containing potassium solubilization zone media.

A-5.4 Spread it uniformly. Invert the plate and incubate them up to 2 weeks at 28 ± 2 °C.

A-5.5 Count the colonies showing solubilization zones and measure their diameter. Calculate average zone of solubilization in mm.

ANNEX B

(Clause 4.6)

GUIDELINES ON MAINTENANCE AND PREPARATION OF CULTURE AND QUALITY CONTROL AT BROTH STAGE

B-1 MAINTENANCE OF PURE CULTURES

B-1.1 Maintain pure culture of KMBI on the medium as described in **A-2.1** in the form of slants.

B-1.2 Transfer a loopful of pure culture to each of the agar slants aseptically in an inoculation room and incubate at 28 ± 2 °C for three days. Always keep pure culture below 5 °C.

B-2 PREPARATION OF INOCULUM CULTURE AND MASS CULTURE

Inoculum culture and mass culture shall be prepared as described in **D-2** and **D-3** of IS 8268 changing the media composition as mentioned in **A-2.1** of this standard.

B-3 QUALITY CONTROL TEST RECOMMENDED AT BROTH STAGE

B-3.1 Qualitative Test

B-3.1.1 Check for free from visible contaminants by microscope and observing solubilization zones.

B-3.1.2 The pH of bacterial broth shall normally be between 6.5 to 7.

B-3.1.3 Gram staining test shall be carried out as described in **D-4.1.3** and **D-4.1.3.2** of IS 8268.

B-3.2 Quantitative Test

B-3.2.1 Viable Cell Count

ANNEX C

(Clause 5.2.3)

DIRECTIONS FOR USE OF POTASSIUM MOBILIZING BIOFERTILIZERS

C-1 The contents of the packet are sufficient enough to the given area to be broadcast or given seedlings for root dipping depending on the specified crops as denoted on the packet. Mix the inoculants with seeds gently with the minimum amount of water, taking care to avoid damage to seed coat. Dry the inoculated seed under shade over clean dry paper organic bag and sow them immediately.

C-2 In order to solubilize fixed soil phosphate use KMBI for all type of crops. Use before the expiry date and do not expose to direct sunlight or heat.

C-3 KMBI is not a chemical fertilizer, hence do not mix inoculated seeds or KMBI with agro-chemicals.

ANNEX D*(Foreword)***COMMITTEE COMPOSITION**

Soil Quality and Fertilizers Sectional committee, FAD 07

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SHRI RAJPAL
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